

Figure 1

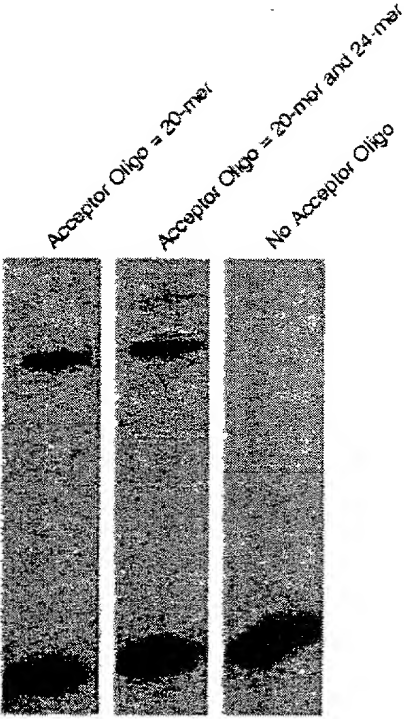


Figure 2

OPS at Topol cleavage site

↓

TCRIBamS: 5' AATTCGCGGCGCGCAAAAAGACTT ~~GATC~~ 3'

TCRIBamAS: 3' GCGCCGGCGGTTTTTCTGAA--CTAG--DMT 5'

Figure 3A

OPS at Topol cleavage site

↓

TCRBstBS: 5' AATTCGCGGCGCGCAAAAAGACTT ~~CG~~ 3'

TCRBstBAS: 3' GCGCCGGCGGTTTTTCTGAA--GC--DMT 5'

Figure 3B

TCRIBamS: 5' AATTCGCGGCGCGCAAAAAGACTTGATC 3'

TCRIBamAS: 3' GCGCCGGCGGTTTTTCTGAACTAG 5'

Figure 3C

GATC removed on topo cleavage to give BamHI compatible 5' overhang

5' AATTCGCGGCGCGCAAAAAGACTTGATCAAGTCTTTTTGCGGCCGCG 5'

3' GCGCCGGCGGTTTTTCTGAACTAGTTCAGAAAAACGCGGCGCTTAA 3'

Figure 3D

Figure 3
A - D

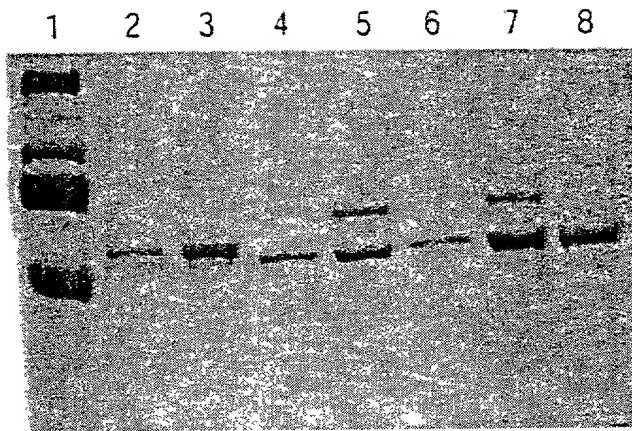


Figure 4

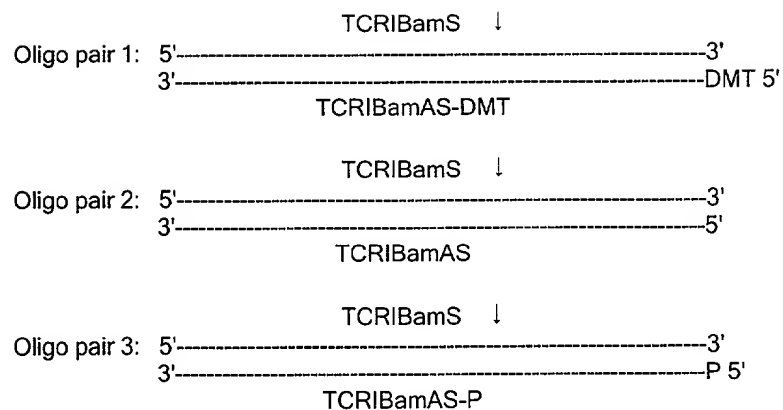


Figure 5A

"↓" indicates site of topo cleavage

Oligo pair 4: TCR1BstBS + TCRABstBAS-DMT

Oligo pair 5: TCR1BstBS + TCRABstBAS

Oligo pair 6: TCR1BstBS + TCRABstBAS-P

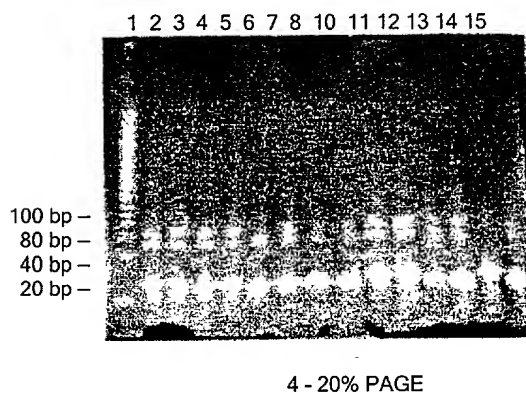


Figure 5B

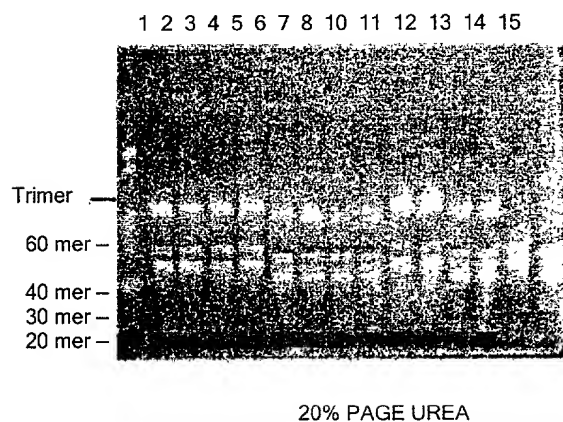
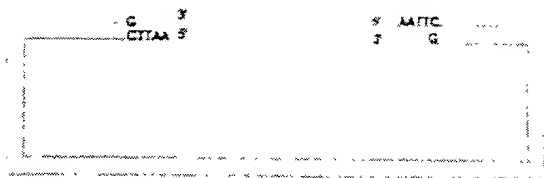


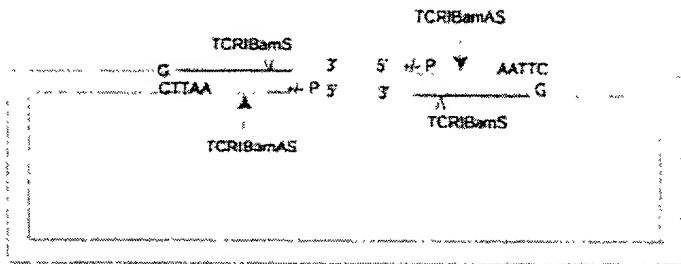
Figure 5C

Figure 5
A - C



pKSII linearized with EcoRI

Figure 6A



GATC overhang left after Topo
cleavage and removal of 4bp
downstream from Topo cleavage site

Adapted pKSII

site of Topo cleavage

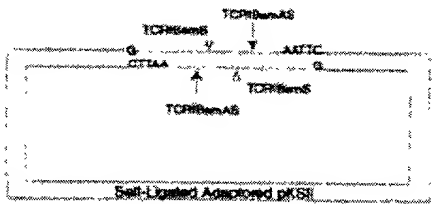


Figure 6B

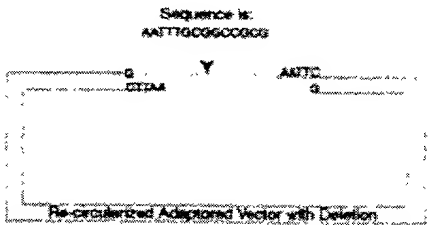


Figure 6C

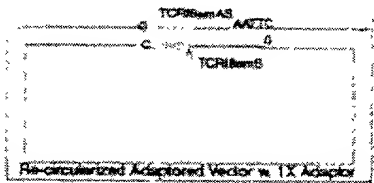


Figure 6D

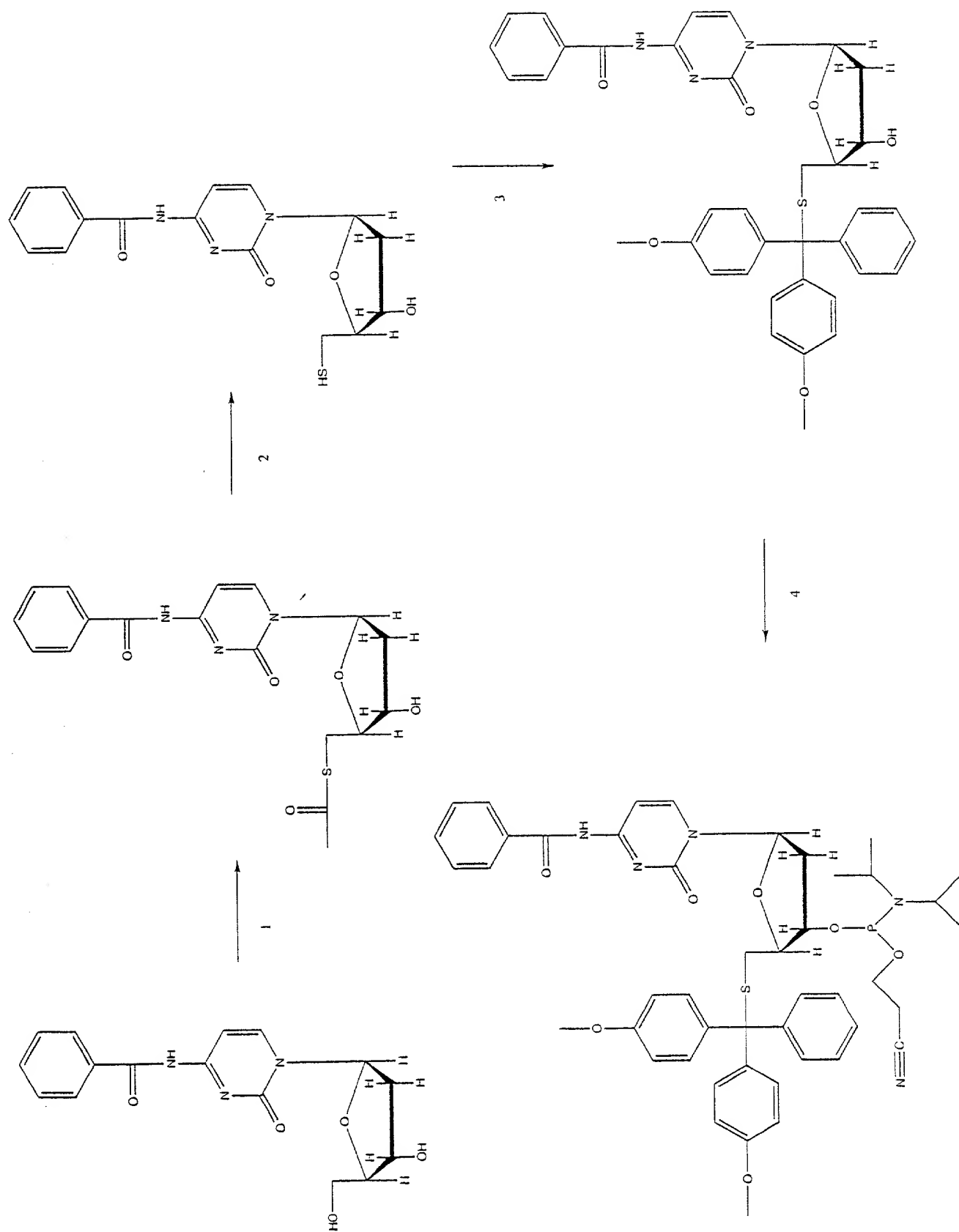


Figure 7A

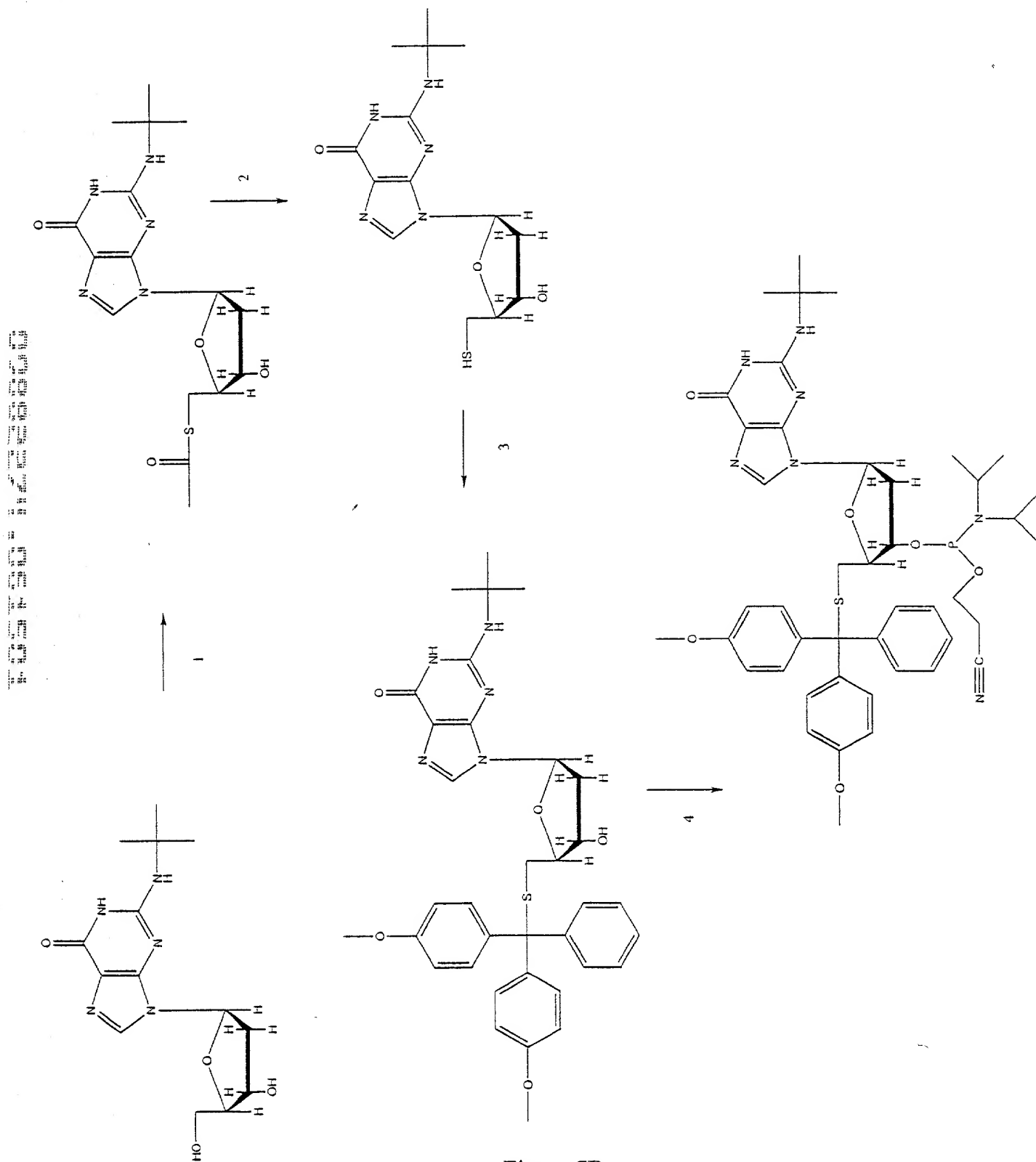


Figure 7B

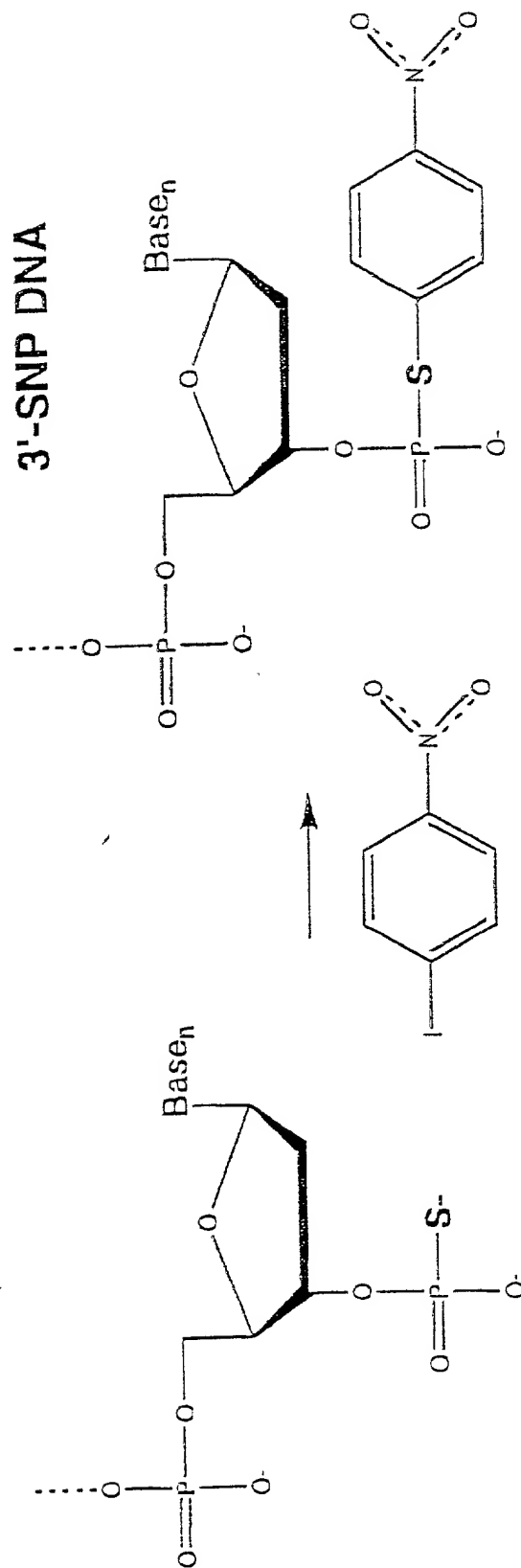


Figure 8